International Journal for Parasitology 45 (2015) 75–83

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Spatial and seasonal factors are key determinants in the aggregation of helminths in their definitive hosts: *Pseudamphistomum truncatum* in otters (*Lutra lutra*)

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ARTICLE INFO

Article history:
Received 18 August 2014
Received in revised form 4 September 2014
Accepted 11 September 2014
Available online 13 November 2014

Keywords:
Parasite aggregation
Seasonality
Parasite transmission
Opisthorchiidae

ABSTRACT

Parasites are typically aggregated within their host populations. The most heavily infected hosts are frequently cited as targets for optimal disease control. Yet a heavily infected individual is not necessarily highly infective and does not automatically contribute a higher proportion of infective parasitic stages than a host with fewer parasites. Here, *Pseudamphistomum truncatum* (Opisthorchiida) parasitic infection within the definitive otter host (*Lutra lutra*) is used as a model system. The hypothesis tested is that variation in parasite abundance, aggregation and egg production (fecundity, as a proxy of host infectivity) can be explained by abiotic (season and region) or biotic (host age, sex and body condition) factors. Parasite abundance was affected most strongly by the biotic factors of age and body condition, such that adults and otters with a higher condition index had heavier infections than sub-adults or those with a lower condition index, whilst there were no significant differences in parasite abundance among the seasons, regions (ecological regions defined by river catchment boundaries) or host sexes. Conversely, parasite aggregation was affected most strongly by the abiotic factors of season and region, which were supported by four different measures of parasite aggregation (the corrected moment estimate *k*, Taylor's Power Law, the Index of Discrepancy *D*, and Boulinier's *J*). *Pseudamphistomum truncatum* was highly aggregated within otters, with aggregation stronger in the Midlands (England) and Wales than in the southwestern region of the United Kingdom. Overall, more parasites were found in fewer hosts during the summer, which coincides with the summer peak in parasite fecundity. Combined, these data suggest that (i) few otters carry the majority of *P. truncatum* parasites and that there are more infective stages (eggs) produced during summer; and (ii) abiotic factors are most influential when describing parasite aggregation whilst biotic factors have a greater role in defining parasite abundance. Together, parasite abundance, aggregation and fecundity can help predict which hosts make the largest contribution to the spread of infectious diseases.

1. Introduction

Patterns of parasite intensity vary widely across a host population (Anderson and May, 1991; Scott and Smith, 1994) and generally parasite distributions tend to be aggregated, a pattern best fit by a negative binomial distribution (Shaw and Dobson, 1995; Shaw et al., 1998; Woolhouse et al., 1997; Galvani and May, 2005). The most infected individuals are frequently cited as key individuals to target for optimal disease control (e.g. Woolhouse et al., 1997; Perkins et al., 2003; Lloyd-Smith et al., 2005; Matthews et al., 2006). High parasite intensities, however, do not necessarily indicate that an individual has a correspondingly high infectivity or transmission potential. Parasite transmission is defined as the probability of both contacting an infective particle/individual and of acquiring that infection. As such, the transmission potential of a host can, in part, be quantified by parasite fecundity, as this measures the number of potential infective stages (Shaw and Dobson, 1995; Shaw et al., 1998). Arguably, hosts that are simultaneously the most infected and harbour parasites that are highly fecund are likely to contribute strongly to the transmission potential of a parasitic disease. There is, however, inherent variation in the reproductive potential of a parasite, affected by both parasite and host age, sex, body condition and host immunity (Kaitala et al., 1997; Luong et al., 2010; Koehler and Poulin, 2012), which may in turn be affected by environmental factors. Identifying the host and environmental factors that are associated with heavily

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http://dx.doi.org/10.1016/j.ijpara.2014.09.004
0020-7519/

infected hosts and/or highly fecund parasites will allow us to focus treatment strategies more specifically. This is vital to prolong the efficacy of drugs, particularly following the emergence of anthelmintic resistance (Laurenson et al., 2013).

Patterns of parasite abundance, aggregation and fecundity have been used frequently to consider the contribution an individual host can make to the spread of disease, although typically each of these variables are considered in isolation (e.g. Madhavi, 1979; Rolfe et al., 1991; Woolhouse et al., 1997; Perkins et al., 2003; Ferrari et al., 2004; Newey et al., 2005). Parasite abundance is related to parasite aggregation (Gregory and Woolhouse, 1993). If parasite abundance, aggregation and fecundity were considered together, it could be possible to identify the factors associated with hosts that are simultaneously the most infected (abundance and aggregation) and infective (fecund), and those individuals could be targeted to optimise treatment strategies (Nielsen, 2012; Laurenson et al., 2013).

In the current study, parasite abundance is defined as the number of individuals of a particular parasite species in or on a single host, regardless of whether or not that host is infected with the parasite (following Bush et al., 1997). An individual with a high abundance could be considered more functionally important with regards to parasite transmission potential than one with a low abundance (or no parasites), such that treatment of heavily infected individuals could lead to a disproportionately high reduction in the parasite population (see Woolhouse et al., 1997; Perkins et al., 2003; Ferrari et al., 2004; Skorping and Jensen, 2004; Magalhaes et al., 2012). Parasite aggregation is a measure of the extent to which parasites are scattered unevenly among available hosts (Shaw and Dobson, 1995; Poulin, 1996a; Shaw et al., 1998). If a pattern exists where the majority of parasites are found within a minority of the hosts then there is potential to isolate and treat those heavily infected few (see Dobson et al., 1992; Hudson et al., 1992; Poulin and Morand, 2000; Newey et al., 2005; Heylen and Matthysen, 2011).

Parasite fecundity is the reproductive potential of a parasite and can be approximated, for helminths, using in utero egg counts (Stear et al., 1995; Richards and Lewis, 2001) or faecal egg counts. The latter represents the quantity of infective particles that may be released into the environment (Madhavi, 1979; Rolfe et al., 1991) and can be used to measure the rate of egg release per unit of time over the reproductive lifetime of the parasite (e.g. Schleppe and Goater, 2004). However, eggs are often shed in the host faeces sporadically (Tinsley, 1983), causing issues for quantification of shed particles if samples are taken infrequently (see Wilson et al., 2001). In utero egg counts for parasites with an elongated uterus may provide an alternative option for estimating reproductive output where it is not possible to quantify parasite transmission potential via faecal egg counts.

In this study, we quantify parasite abundance and aggregation across a host population and estimate parasite fecundity using in utero egg counts of *Pseudamphistomum truncatum* (Trematoda, Opisthorchiidae) infections in otters, *Lutra lutra* (see Sherrard-Smith et al., 2009). *Pseudamphistomum truncatum* is a mammalian biliary parasite with a three host life cycle that is trophically transmitted to otters via the consumption of a second intermediate host, cyprinid fish. The parasite matures in the otter and, typical of digeneans, accumulates egg capsules in an elongated uterus. Eggs are deposited into the environment with faeces and ingested by snails, which subsequently release cercariae that can encyst on the cyprinid fish. The parasite is considered to damage the otter biliary system, particularly when heavy infections occur (Simpson et al., 2005, 2009). The otter-*P. truncatum* system is used here to examine two questions. (i) Are patterns of parasite abundance and aggregation related to seasonality and geographic location and/or host sex, age and body condition? (ii) Is parasite fecundity higher in specific groups of hosts; in other words, is it possible to identify individuals that have the potential to disseminate significantly more infective stages than others? Using a helminth population from a long-term survey of wild animal cadavers as a model system, parasite abundance, aggregation and fecundity are considered within a single system, allowing identification of the factors that might contribute disproportionately to parasite transmission potential.

2. Materials and methods

2.1. Sample collection

Road-killed otters, *L. lutra* ($N = 516$ of which 72 were infected) were collected from across England and Wales, United Kingdom (UK) as part of a national monitoring scheme from 2004 to 2010 (Cardiff University Otter Project (CUOP), see Chadwick et al., 2011). Gall bladders were removed and examined for the presence of biliary parasites. *Pseudamphistomum truncatum* were identified morphologically (following Yamaguti, 1971). The location of each otter was assigned to an ecologically relevant Environment Agency region of the UK (Anglian Region, Wales, Southwest, Midlands, Northwest, Northeast, South of England, Thames, UK) delineated by river catchment drainage boundaries (see Sherrard-Smith et al., 2009), which to a large extent mirrors genetic structure of otters in the UK (Hobbs et al., 2011). Month and year of host death, sex, age class (adult or sub-adult), length and body mass were recorded. A body condition (BC) index for otters was calculated based on otter length and mass (Kruuk et al., 1987). Body condition, previously referred to as K (see Kruuk et al., 1987; Sherrard-Smith et al., 2009), is here renamed BC, to distinguish it from the negative binomial dispersion parameter k, used in this study to measure parasite aggregation. BC is measured by the following equation:

$$\mathrm{BC} = \mathrm{weight(kg)}/[a^{*}\mathrm{length}^{n}] \quad (1)$$

where $a = 5.02$ and $n = 2.33$ for females, and $a = 5.87$ and $n = 2.39$ for males (following Kruuk et al., 1987). Body condition was included in analyses of aggregation to allow aggregation to be explored using the same variables as those included in the abundance and fecundity analyses (please see below). The hosts were split into two groups; this split was arbitrary but allowed a comparison to be made between hosts with a high condition index ($\mathrm{BC} \geqslant 1$) and those hosts with a low condition index ($\mathrm{BC} < 1$). Hosts were also categorised by age-class as 'adult' or 'sub-adult', with sub-adults defined as males with a baculum length of less than 60 mm and females that were not yet reproductively active (see Sherrard-Smith and Chadwick, 2010). Seasons were defined as spring: March, April and May; summer: June, July and August; autumn: September, October and November; and winter: December, January and February.

To assess whether there were distinct differences in parasite abundance due to an abiotic factor (season of host death (winter, spring, summer or autumn) and geographic location (restricted to regions with five or more infected otters; Wales, Midlands or Southwest)) or biotic factors (age-class (sub-adult or adult), sex (male or female) and condition (good or poor) (see Table 1 for sample sizes)), Generalised Linear Models (GLMs, with negative binomial error distributions) were fitted with year included as a random effect to account for inter-annual differences. Otter condition was significantly lower in summer than winter (ANOVA: $F_{3,514} = 4.52$, $P < 0.005$), consistent with the original study on otter condition (Kruuk et al., 1987) so that the BC index was considered independently from season in models fitted to parasite abundance, aggregation and fecundity. To compare the degree of aggregation of parasites within the population, differences between the

Table 1
Summary statistics describing four measures of aggregation for a host–parasite interaction between the otter, *Lutra lutra*, and *Pseudamphistomum truncatum*. Biotic or abiotic sub-groups of the host population are differentiated by season, region, host age class, sex and host condition. Bold type-face refers to the most aggregated population among factors and significant differences among sub-groups are identified.

Population	Sub-group	Prevalence (%)	Mean abundance	Variance	Hosts (*N*)	Infected Hosts (*N*)	Measures of parasite aggregation			
							k parameter	Index of Discrepancy *D*	Variance-to-mean ratio (VMR)	J_j (95%CI)
Season	1 Summer	15.9	5.6	1332.1	69	11	**0.0089**	**0.96**^{2,3,4***}	4.185	**42.210 (18.9–101.8)**
	2 Autumn	12.6	2.2	198.3	167	21	0.0187	0.95^{3,4***}	**6.695**	40.372 (18.1–82.2)^{1}
	3 Winter	13.8	5.1	575.3	167	23	0.0390	0.94	3.917	22.140 (11.9–47.2)^{12,***}
	4 Spring	15.0	3.9	257.0	113	17	0.0506	0.94	4.089	16.701 (6.8–42.7) ^{1,2***}
Region	1 Wales	17.4	4.5	702.8	144	25	0.0224	0.94^{3***}	**4.341**	**33.931 (14.1–71.2)**
	2 Midlands (England)	16.2	4.1	365.9	37	6	**0.0199**^{1,3}	**0.94**[a]	4.158	20.860 (7.2–39.6)^{1***}
	3 Southwest (England)	34.0	11.5	1194.5	106	36	0.1019	0.89	2.903	8.9052 (4.6–16.2)^{1,2***}
Age	1 Adults	17.5	5.6	780.3	285	50	0.0377	**0.96**	3.846	24.220 (14.5–52.0)
	2 Sub-adults	10.6	2.1	122.1	207	22	**0.0307**	0.95	**6.615**	**28.077 (14.1–65.1)****
Sex	1 Females	12.5	4.5	745.5	232	29	**0.0225**	**0.96*****	4.423	**37.144 (16.1–71.6)**
	2 Males	15.1	3.5	269.9	284	43	0.0433	0.95	**4.437**	21.323 (12.3–43.7)***
Condition	1 Low condition index	13.0	1.7	60.2	192	25	0.0415	**0.96*****	**8.122**	21.370 (11.3–46.8)
	2 High condition index	14.5	5.3	729.3	324	47	**0.0358***	0.95	3.949	**25.644 (14.7–43.1)*****
Total population		13.9	3.9		516	72				

Superscript numbers represent which sub-groups were significantly different e.g. Poulin's Index of Discrepancy *D* indicated that summer was significantly different to autumn, winter and spring.
Significance of statistical tests are represented by $P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
For factors with more than two sub-groups, numbers are used to indicate the sub-group that differs from the current comparison (e.g. significant difference for J_j between summer and autumn populations $P < 0.1$, summer and winter $P < 0.001$).
CI, confidence interval.
[a] Sample size too small for comparison.

categorical abiotic and biotic factors were explored independently using four different metrics of aggregation. Again, regional differences were tested only for regions with five or more infected otters.

2.2. Aggregation parameters

Several indices have been developed previously to explore the degree of parasite aggregation within a host population, including the corrected moment estimate *k*, Taylor's Power Law, Poulin's Index of Discrepancy (*D*) and Boulinier's *J*, and these can be used to identify groups of infected individuals. Comparing aggregation between populations is generally challenging (Wilson et al., 2001). The corrected moment estimate of *k* is the most commonly used measure of parasite aggregation and it is widely accepted, particularly because in comparisons of various indices (variance to mean ratio, coefficient of variation, moment estimate of *k* and corrected moment estimate of *k*), the corrected moment estimate of *k* was found to vary least with different mean parasite loads and sample sizes (Gregory and Woolhouse, 1993). Regardless, there is still an element of co-variance with the mean when using the *k* parameter (see Gregory and Woolhouse, 1993).

Taylor's Power Law (Taylor and Taylor, 1977) relates the between sample variance to the overall mean abundance of a given sample of organisms and is most useful when a group of samples or populations are available for consideration (see Wilson et al., 2001). The Index of Discrepancy (*D*) is more host-centric than *k* or Taylor's Power Law and less sensitive to the distribution of parasites (Poulin, 1993). The *D* index can vary between zero (no aggregation) and one (all parasites are theoretically within a single host) and thus has the potential to compare datasets with varying prevalences and mean parasite loads. Equally, Boulinier's *J* considers the relative clustering of parasites, by calculating the likelihood of additional parasites occupying a host, if that host is already infected by any given parasite (Boulinier et al., 1996). As such, Boulinier's *J* quantifies parasite aggregation from an individual host perspective rather than a population level metric and has the advantage that, unlike the *k* parameter, it is not biased for small sample sizes (Gregory and Woolhouse, 1993; Boulinier et al., 1996). Consequently, comparing aggregation between sub-samples of hosts is problematic because no single measure of aggregation is entirely reliable and aggregation indices vary according to sample size and abundance. To achieve confidence in the patterns observed, four methods are applied to quantify parasite aggregation across abiotic and biotic factors and see if there is congruence between multiple measures.

2.2.1. The corrected moment estimate (*k*)

The corrected moment estimate (*k*) of the negative binomial distribution quantifies an increase in parasite aggregation, for a constant mean, with a diminishing *k* value, i.e. it is an inverse measure of aggregation. For example, if the parasite population is highly aggregated in the host population, *k* tends towards a theoretical limit of zero (where all parasites are concentrated within a single host). Conversely, for the same given mean parasite abundance, as *k* increases, parasite aggregation decreases so that the distribution tends towards a Poisson (random) distribution followed by a positive binomial as *k* increases to infinity. The corrected moment estimate *k* is calculated using the mean parasite abundance *x*, variance σ^2, and sample size *N* of the given population:

$$k = (x^2 - \sigma^2/N)/(\sigma^2 - x) \quad (2)$$

The frequency distributions within host sub-groups were compared to identify differences in *k* using GLMs with negative binomial error distributions.

2.2.2. Taylor's Power Law

To examine differences in parasite aggregation within host sub-groups, the departure of the variance of the number of parasites per host among categories from a random distribution was quantified, and then comparable populations were examined by using a bootstrapping technique to calculate Taylor's Power Law parameter *b* following Boag et al. (2001). Briefly, 50 parasite counts from

each population were sampled at random (with replacement) to calculate the log (mean + 1) and log (variance + 1). This was replicated 50 times giving an estimate of b (the gradient of the linear regression of log (variance + 1) onto log (mean + 1)). This process was repeated 100 times to calculate the S.E. for the intercept (a) and b. Where possible, statistical comparisons between groups (e.g. spring, summer, autumn and winter) were performed using GLMs (Table 2). In some cases, the large number of zeros in the analysis led to b estimates of zero and statistical comparisons were not possible using this method. A simple two-sample Kolmogorov–Smirnov test was then applied to test whether samples had the same distributions.

2.2.3. The Index of Discrepancy (D)

Poulin's Index of Discrepancy (D) quantifies the degree of inequality between the observed distribution and a hypothetical distribution where parasites are distributed equally among hosts. Here, zero represents perfect equality and one implies all of the parasites are aggregated within a single host. To quantify D, hosts were ranked from the most to the least infected individuals (including those without infection). It was then possible to calculate the proportion of parasites associated with any given percentage of infected hosts and D was calculated using:

$$D = 1 - \frac{2\sum_{i=1}^{N}\left(\sum_{j=1}^{i} X_j\right)}{x * N(N+1)} \quad (3)$$

where N = total host population, X is the number of parasites in host j, and x is the mean number of parasites (see Poulin, 1993).

To examine whether parasites are distributed differently between abiotic (season and region) or biotic (age, sex and condition) factors using D, a GLM, binomial error distribution, was fitted to the number of parasites per given proportion of hosts (0.01–0.99), bound to the total number of parasites in the associated factor for each factor independently, thus allowing comparison of the regression lines that describe each category (factors: season or geographic region, age, sex or condition).

2.2.4. Boulinier's 'J'

An alternative method to examine parasite aggregation within and among hierarchical scales was proposed by Boulinier et al. (1996). This method was developed to look at spatial scale impacts on parasite aggregation. The aggregation index, J_j, measures the increase in the expected number of other parasites on a host for any given parasite:

$$J_j = 1/n_j \frac{\sum_{i=1}^{n_j} x_{ij}(x_{ij}-1)}{\left\{X_j\left(X_j - 1 + \left(\frac{n_j - 1}{n_j}\right)^2\right)\right\}} - 1 \quad (4)$$

where n_j is the number of hosts in a given population j; x_{ij} is the corresponding number of parasites in individual, i, from population j; and X_j is the mean abundance for the total population j.

To examine whether the J_j index indicated significantly different aggregation patterns between comparative sub-groups (e.g. males versus females), a bootstrapping method was devised to generate confidence intervals (CIs) for a given population: 100 independent data sets with negative binomial distributions were simulated, parameterised by the respective mean, k and host population sizes of each sub-sample (e.g. female hosts). J_j was calculated for each of these 100 generated datasets and then the J_j data were ranked from smallest to largest so that the 3rd and 98th values could represent the lower and upper 95% CI, respectively. A posteriori testing in ANOVA was then applied to the simulated J_j data; if the CIs overlapped with the J_j for the real data then there was no significant difference between parasite aggregations among sub-groups. Conversely, where CIs did not overlap the J_j for a given sub-sample, a significant difference between sub-groups of the host population was recorded.

2.2.5. Comparing aggregation parameters

Here, a comparison of the degree of parasite aggregation among sub-groups of a host population was undertaken using four different measures of parasite aggregation. The analysis of J_j is promising because this measure of aggregation does not vary with small sample sizes (a limitation of k). An aggregation estimate based on a small sample size, regardless of the method used, is likely to underestimate parasite aggregation because heavy infections are rare and therefore most likely to be observed only when sample sizes are large (Poulin, 1993, 1996b). The J_i approach asks a slightly different question to k, Taylor's Power Law and D. For any given parasite, at the scale of aggregation among hosts, J_j asks: what is the expected increase in the number of additional parasites on a given host relative to a case where parasites are distributed randomly across the host population? It is appropriate, therefore, to consider a range of estimates of parasite aggregation to provide a comprehensive insight into a given system.

Table 2
Comparison of the degree of aggregation across populations using Taylor's Power Law where appropriate and where necessary the non-parametric two-sample Kolmogorov–Smirnov test is used to test whether two samples are drawn from the same distribution.

Factor	Sub-population	Taylor's Power Law b (CI)	a (95% CI)	Statistical difference
Season	Summer	0.272 (0–3.17)	0.037 (0–0.52)	$F = 7.58$, df = 3, $P < 0.001$
	Spring	0.093 (0–2.40)	0.046 (0–1.10)	Summer vs winter $t = 2.586$, $P < 0.01$
	Autumn	0.017 (0–0.00)	0.001 (0–0)	Summer vs spring $t = 2.174$, $P < 0.05$
	Winter	0.035 (0–0.00)	0.020 (0–0)	
Region	Southwest (England)	1.972 (1.69 – 2.23)	1.955 (1.27–2.72)	$F = 1340$, df = 2, $P < 0.001$
	Wales	0.031 (0–0.00)	0.003 (0–0)	Southwest vs Wales $P < 0.001$
	Midlands (England)[a]	0.051 (0–0.00)	0.000 (0–0)	Southwest vs Midlands $P < 0.001$
Age	Adults	0.003 (0–0.00)	0.000 (0–0)	Non-parametric K–S test:
	Sub-adults	0.004 (0–0.00)	0.000 (0–0)	$D = 0.070$, $P = 0.605$
Sex	Females	0.050 (0–0.00)	0.009 (0–0)	Non-parametric K–S test:
	Males	0.023 (0–0.00)	0.007 (0–0)	$D = 0.026$, $P = 0.999$
Condition	High	0.273 (0–2.82)	0.104 (0–1.22)	$F = 21.85$, df = 1, $P < 0.001$
	Low	0.119 (0–3.39)	0.002 (0–0)	

CI, confidence interval; df, degrees of freedom.
[a] The Midlands Region $N = 37$, therefore the parasite counts was reduced to 25 for this population.

2.3. Parasite fecundity

Parasite fecundity is the lifetime egg output, which for trematodes is measured as the rate of egg release (e.g., number of eggs per day) multiplied by the length of the patency period. Given the use of host samples that were opportunistically collected, the majority as road-killed animals, it was not possible to measure the rate of egg release and instead, the number of reproductive units (eggs) within the uterus of each parasite was used as a rough proxy for parasite fecundity. This method has been used previously as a fecundity estimate, particularly where faecal egg count data was unavailable (e.g. Richards and Lewis, 2001; Luong et al., 2010). In the current study, the state of decomposition of hosts (opportunistically collected road-kill) prevented the use of all 72 infected hosts for in utero analysis of the parasites. As such, to examine the parasite fecundity across different abiotic and biotic factors, 35 infected hosts with a total of 255 *P. truncatum* were used. From these 35 hosts (255 parasites), a subset of 119 parasites from 19 hosts were flat-fixed and measured (length, width and area) before storage in 70% ethanol prior to egg counts, allowing us to test whether parasite fecundity is confounded by parasite size. A photographic method was developed to count in utero fecundity. Each parasite was teased apart in 2 ml of distilled water within an adapted microscope slide with a 2 mm high rim surrounding a 15 mm^2 central arena. The slide was scanned at 400× magnification and approximately 500 images were taken from each slide to cover the 15 mm^2 area in fine detail. These images were knitted together without overlap of adjacent images. These images were then screened manually, counting all eggs touching or crossing the top and left edge, and ignoring those on the bottom and right edge of each image, to avoid counting eggs twice. There data were used to test how mean parasite fecundity varied with abiotic and biotic variables (mixed-effects GLM with Gaussian error distribution and identity link function). All statistical analyses were conducted using the package R, version 12.1 (R Development Core Team, 2010. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria) and final models were chosen using the Akaike Index Criterion (AIC) method, dropping an explanatory term if the change in AIC was >2. $P < 0.05$ was considered significant.

3. Results

3.1. Parasite abundance

Pseudamphistomum truncatum infects 13% of UK otters ($N = 72$ infected out of 516 otters, mean abundance 3.9 ± 0.97, mean intensity = 28.3 ± 6.24, range = 1–302). Host age and geographic region were associated with differences in parasite abundance (GLM, negative binomial error distribution: $\chi^2_{10,507} = 18.3$, $R^2_{adj} = 0.74$, $P < 0.001$) such that adults (mean infection = 5.6 compared with sub-adults = 2.1) have higher infections (Likelihood-Ratio Test (LRT) = 27.97, deviance = 180.8, $P < 0.001$). As expected, based on previous spatial analyses (Sherrard-Smith et al., 2009), heavier infections were associated with otters in Wales (4.5 ± 2.2 S.E.), the Midlands (4.1 ± 3.1) and the Southwest (mean infection = 11.4 ± 3.3) than elsewhere (LRT = 137.2, deviance = 299.7, $P < 0.0001$). There were no significant differences in parasite abundance with host sex or condition, or season or year of death (not included in final model).

3.2. Parasite aggregation

Analysis of the aggregation index k found no seasonal differences (GLM negative binomial error distribution, $P > 0.1$) but parasites were more aggregated in the Midlands region compared with the Southwest ($\chi^2_{2,25} = 1.669$, S.E. = 0.773, $P = 0.095$) and Wales ($\chi^2_{2,25} = 1.669$, SE = 0.773, $P = 0.091$), but only at the 90% confidence level. There was no significant difference in k between host ages or sexes (GLM negative binomial error distribution, $P > 0.1$ for all cases, Table 1). Parasites were significantly more aggregated in poor condition hosts compared with those in good condition (GLM negative binomial error distribution: $\chi^2_{1,514} = 1.875$, S.E. = 0.479, $P = 0.044$).

The analysis of Taylor's Power Law showed parasites from hosts were more aggregated in summer than in either winter ($P < 0.01$, see Table 2) or spring ($P < 0.05$). Equally, parasites were more dispersed in the Southwest population compared with either Wales ($P < 0.001$) or the Midlands ($P < 0.001$). Parasites were not aggregated differently between host age or sex classes (Table 2) but parasites were more aggregated in hosts in poor condition compared with those in good condition ($P < 0.001$).

Using the D index analysis, parasites were significantly more aggregated during the summer ($D = 0.96$) than during other seasons (GLM: $\chi^2_{3,83} = 9.765$, $P < 0.001$) whilst parasites were more evenly dispersed across the otter population in winter ($D = 0.94$) and spring ($D = 0.94$) than during autumn ($D = 0.95$) (GLM: autumn versus winter $\chi^2_{3,83} = 4.803$, $P < 0.001$; autumn versus spring $\chi^2_{3,83} = 7.184$, $P < 0.001$). Only six otters out of 46 were infected in the Midlands region (see Table 1), preventing detailed analysis of any patterns that might arise between this region and others. Parasites in otters from the Southwest were, however, less aggregated than those in otters from Wales (GLM: $\chi^2_{2,62} = 1.404$, $P < 0.001$). There were no differences between host age classes (GLM: $\chi^2_{1,41}$=0.798, $P = 0.425$) but parasites were more aggregated in female otters than males (GLM: $\chi^2_{1,41} = 7.529$, $P < 0.001$) and more aggregated in poor condition otters than those in good condition (GLM: $\chi^2_{1,41} = 3.803$, $P < 0.001$).

The J_j index found significant differences among all abiotic and biotic factors. Parasites in otters collected during summer ($J_j = 42.2$; 95% CI = 18.9–101.8) and autumn ($J_j = 40.4$; 95% CI = 18.1–82.2) were more aggregated than those from winter ($J_j = 22.1$; 95% CI = 11.9–47.2) and spring ($J_j = 16.7$; 95% CI = 6.8–42.7). The 95% CIs overlapped least within the regional factor, indicating that parasites in otters from the Southwest ($J_j = 8.9$; 95% CI = 4.6–16.2) were distributed more evenly among the host population than those in either Wales ($J_j = 33.9$; 95% CI = 14.1–71.2) or the Midlands ($J_j = 20.9$; 95% CI = 7.2–39.6) (Fig. 1). Parasite aggregation was greater in sub-adults ($J_j = 24.2$; 95% CI = 14.5–52.0), female otters ($J_j = 37.1$; 95% CI = 16.1–71.6) and those in good condition ($J_j = 25.6$; 95% CI = 14.7–43.1) than adults ($J_j = 24.2$; 95% CI = 14.5–52.0), male otters ($J_j = 21.3$; 95% CI = 12.3–43.7) or those in poor condition ($J_j = 21.4$; 95% CI = 11.3–46.8) where a higher J_j value is indicative of greater aggregation among the host population (Table 1).

In summary, three out of four measures of aggregation found significant differences between the seasons; parasites aggregated in fewer hosts during summer ($k = 0.008$, $D = 0.96$, $J_j = 42.210$, Table 1) than in spring. Only Taylor's Power Law indicated aggregation was strongest in autumn (Table 1). For all measures of aggregation, parasites in otters were more aggregated in Wales ($k = 0.025$, Taylor's Power Law $b = 0.031$, $D = 0.94$, $J_j = 33.931$) and the Midlands ($k = 0.016$, Taylor's Power Law $b = 0.051$, $D = 0.94$, $J_j = 20.860$) than in the Southwest otter populations ($k = 0.097$, Taylor's Power Law $b = 1.972$, $D = 0.89$, $J_j = 8.905$). The aggregation indices were similar for the host ages and sexes, indicating that these factors do not have a significant impact on parasite aggregation (Tables 1 and 2). There was disagreement for condition with the k parameter, Taylor's Power Law and J_j, indicating the parasites were more aggregated in otters with a high condition index whilst D and the variance to mean ratio (VMR) indicated parasites were

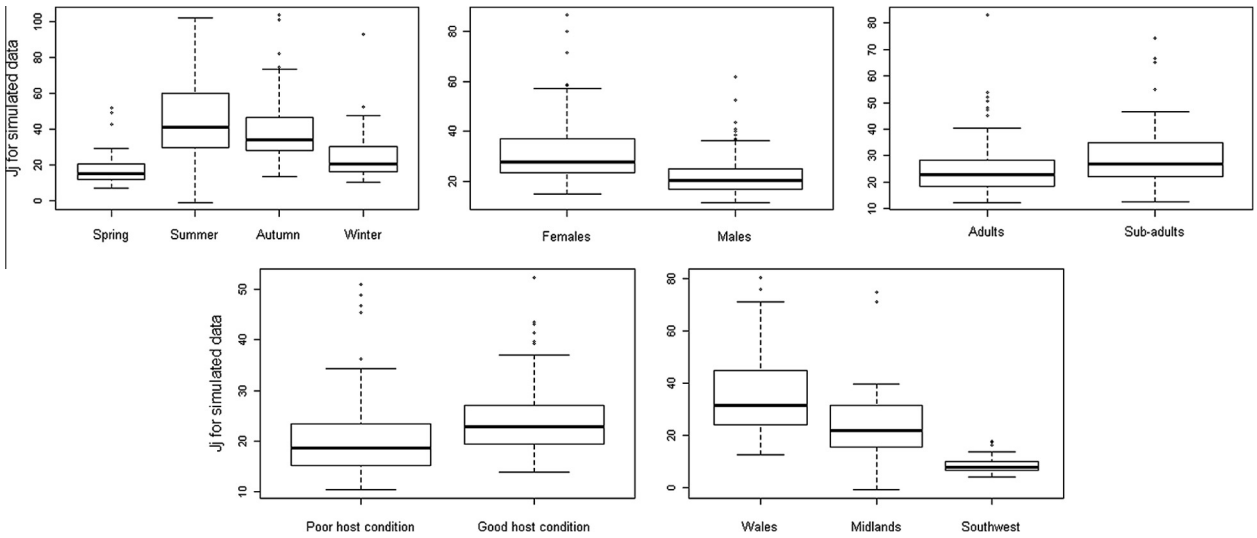

Fig. 1. Comparison of the Boulinier et al. (1996) measures of parasite aggregation (J_j) in otters for 100 simulated populations parameterised on the mean, sample size and corrected moment estimate k for each sample, respectively. A higher J_j indicates greater aggregation. The range of the J_j for the sub-groups in each factor (season, sex, age, host condition and geographic region of the United Kingdom (Wales, the Midlands (England) and Southwest (England) are represented.

more aggregated in otters with a low condition index (Tables 1 and 2).

3.3. Parasite fecundity

Mean intensity for the 35 sampled hosts included in the fecundity analysis was 48.3 (range = 1–302, S.D. = 72.4). Fecundity ranged from 351 to 3391 eggs with a mean = 1435 (S.D. = 791.4) for $N = 255$ parasites. Initial analyses to examine whether parasite size is associated with parasite fecundity revealed a positive relationship between parasite length and width, and the number of eggs produced (GLM of parasite length and width fitted to egg count for $F_{115,119} = 44.77$, $P < 0.005$) indicating larger parasites carry more eggs. Parasite length or width were not significantly associated with season (ANOVA, $P > 0.1$ for both cases) and neither parasite fecundity, parasite length nor parasite width were associated with host factors (age, sex and condition), indicating variation among these biotic factors does not explain any observed differences in fecundity. Equally, mean parasite intensity per host did not correlate with mean parasite fecundity per host (LR: $F_{35} = 0.98$, $P = 0.329$), indicating there was no density-dependent effect acting on parasite fecundity. Higher egg counts were associated with spring (mean egg count per parasite, 1627, S.E. ±154.2) and summer (1852, ±91.5) with lower counts in autumn (1422, ±80.6) and winter (1005, ±163.6). However the fecundity of parasites was only statistically significantly higher in summer compared with winter (GLM: $t_{31} = 2.091$, $P < 0.05$) (Fig. 2). Therefore, individuals are potentially most infective during summer. There was no statistically significant relationship between mean egg counts and host age, sex or condition, or the intensity of the parasitic infection (GLM: $F_{14,19} = 0.45$, $P = 0.77$).

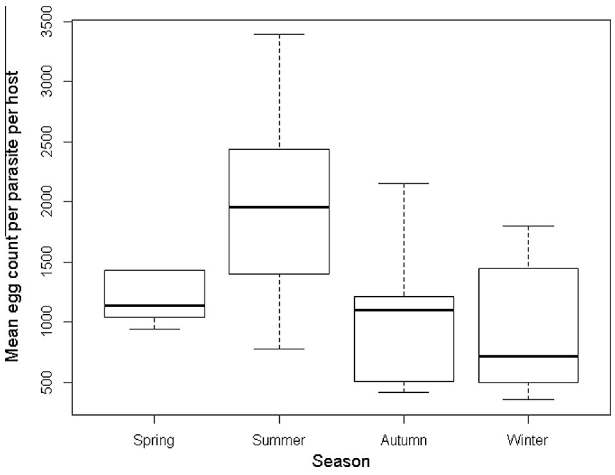

Fig. 2. Differences in *Pseudamphistomum truncatum* fecundity with season; significant difference is highlighted between summer and winter. A significant difference was observed between summer and winter ($P < 0.05$).

4. Discussion

In the current study, abundance, aggregation and fecundity were considered together with the aim of identifying key factors that are associated with the most infected hosts and those with the greatest transmission potential (using fecundity as a proxy). Within our otter-helminth system, biotic factors influenced parasite abundance such that adults and otters in good BC had higher mean helminth abundance than sub-adults or those in poor condition. Conversely, both abiotic and, to a lesser extent, biotic factors explained aggregation and fecundity differences such that *P. truncatum* were most highly aggregated in summer and female hosts and the helminths were producing and/or storing significantly greater numbers of eggs during summer. There were significant geographical differences in parasite aggregation with infections tending to be aggregated most strongly across the otters from Wales and the Midlands, and less strongly in otters from the Southwest. The different aggregation indices all highlighted seasonal and regional differences but the J_j index was most sensitive and additionally indicated differences among host age classes, host sex and condition.

The biotic factors, host age and condition, had a greater impact on parasite abundance than the abiotic factors examined (season and region). The predominantly road-killed otters used in the current study were typically aged between 1 and 2 years (Sherrard-Smith and Chadwick, 2010) and it is likely that the youngest and oldest proportion of the population were under-represented in our sample. Older animals may be particularly important for

parasite studies where infections accumulate with age, such as the trophically transmitted trematodes considered here. Higher mean parasite counts were observed in adult otters compared with sub-adults. If heavily infected older otters were under-represented then parasite aggregation may also have been underestimated, reducing the degree of aggregation observed. This is a recognised limitation of the current study and has been highlighted in previous studies on parasite aggregation when sample sizes were small (see Wilson et al., 2001). There were no differences in aggregation between host age classes in the current study. Although not always present (see Henricson, 1977; Wilson et al., 2001; Heylen and Matthysen, 2011), system-specific age group differences have been observed in parasite aggregation (Quinnell et al., 1995). The warble fly, *Hypoderma bovis*, is most aggregated in younger cohorts of cattle (see Breyev and Minar, 1976), possibly because the fly actively searches for its host and thus differences in the attractiveness of various age classes may dictate parasite aggregation. In the current study, the transmission route of the trematodes to adult or sub-adult otters is identical and may explain the observed absence of an age difference in parasite aggregation.

Differences in parasite aggregation have been observed previously between: (i) native and non-native hosts (Hodasi, 1969); (ii) different causes of host death (Hudson et al., 1992); (iii) different parasite body sizes (Poulin and Morand, 2000); (iv) different host body sizes (Poulin, 2013); (v) across seasons (Dronen, 1978; Newey et al., 2005). Here, across the four different methods used to quantify parasite aggregation in the host population, it was observed that the abiotic factors, season and region, had the greatest influence on the degree of parasite aggregation (Table 1). Season and condition are likely confounded, however, such that otters have a lower BC index during summer and a higher one through winter (our data and Kruuk et al., 1987). There is duality in this interaction; otters have a generally lower BC index during summer. This may be a consequence of sub-lethal effects of seasonally aggregated parasites or otters with a lower BC index may be more susceptible and more likely to develop heavier infections leading to the high parasite aggregation. Three out of four measures of aggregation (k-parameter estimates, D and J_j) found significant differences between the seasons, indicating parasites aggregated in fewer hosts in the summer and were more over-dispersed in spring. Equally, for all measures of aggregation, parasites in otters from Wales and the Midlands were more aggregated than those in the Southwest otter populations. A key issue that is associated with the negative binomial k parameter, but which also applies to the other aggregation measures explored here, is that introducing interventions assumes that parasite aggregation remains constant (see Yakob et al., 2014). It is clear from the discrepancy in parasite aggregation in the otters across seasons that these systems are dynamic and a single measure of k parameter estimates during a given year is perhaps not sufficient to explore the success or failure of interventions.

Seasonal patterns in parasite aggregation have been observed for *Trichostrongylus retortaeformis* in mountain hares (Newey et al., 2005). This lagomorph-helminth system exhibited increased parasite aggregation in winter, which was attributed to reduced parasite transmission and infection across winter months. The *P. truncatum* life cycle is more complex than that of *T. retortaeformis* because the former requires intermediate hosts. Currently, we do not know whether there is a peak season during which the majority of otters become infected due to the probable long life span of *P. truncatum* and opportunistic sampling of otters (road-kills).

In our study system, parasite aggregation differences were observed between UK regions (Wales, Midlands, Southwest) and show that there is heterogeneity in host–parasite populations across the UK and factors that are driving aggregation within a region act to different degrees. The regional differences observed may relate to host exposure (the probability of encountering a parasite), especially given that the parasite studied infects intermediate hosts that may have spatially heterogeneous distributions themselves. Further, the transmission route of infections (e.g. direct versus trophic transmission) has been shown to contribute to parasite aggregation patterns (Shaw and Dobson, 1995) and could be further accentuated within the otter population if certain otters were preferentially consuming infected species i.e. species of food organisms hosting 'infective' stages of the parasites.

The aggregation indices were similar for the host sexes and age classes, indicating that the examined biotic factors are less important than abiotic factors in determining differences in the degree of *P. truncatum* aggregation in otters. However, both D and J_j identified differences (95% CI) in aggregation between the sexes, suggesting aggregation was stronger in female otters than in males. There are subtle differences in the ecology of otters between the sexes that may account for greater aggregation among female otters. Behaviourally, the larger male otters tend to have larger geographic ranges than females (Kruuk and Moorhouse, 1991) so males are more likely to be exposed to parasites than more locally restricted females (Nunn et al., 2003; Vitone et al., 2004). However, parasites tend to have geographically patchy distributions (e.g. Jokela and Lively, 1995) so that females are only likely to become infected if resident at a parasite-rich location. As a result, across the population, fewer females will be infected but these may harbour heavier infections, perhaps resulting in the observed slightly higher parasite aggregation in females. Keymer and Anderson (1979) demonstrated that within an arena with uniform distribution of infective stages, naïve beetles became infected to different degrees such that variation in host behaviour or immunity must contribute to the overall parasite distribution across a population. It is very likely that factors not measured in this study, such as host behaviour and immunity differences, as well as host and parasite genetics, are also acting on the trematode aggregation across the otter population.

Otters are a protected species in the UK and elsewhere in Europe (International Union for Conservation of Nature (IUCN) Red List of Threatened Species, 04 December 2013; http://www.iucnredlist.org/) and the presence of parasites with the potential to cause morbidity (see Simpson et al., 2005, 2009) is cause for concern. The life cycle of *P. truncatum* includes two intermediate hosts; both *Bithynia* snails and cyprinid fish are highly abundant in freshwater environments in the UK and across Europe so there is potential for the maintenance of this parasite regardless of any intervention to protect the definitive host. A potential reason behind the recent identification of *P. truncatum* might be the establishment of mink (*Neovison vison*) – a second viable definitive host – increasing the abundance of *P. truncatum* (Sherrard-Smith et al., 2014). The likelihood of encountering a definitive host will be associated with parasite aggregation, abundance and fecundity in intermediate hosts and careful consideration of these patterns in earlier life stages is needed to complement our understanding of distributions in the definitive hosts.

In conclusion, the current study indicates that differences in parasite abundance are explained by both biotic (age) and abiotic (region) factors, whilst parasite aggregation patterns and parasite fecundity are better explained by abiotic factors (season and region). Seasonality plays a fundamental role in the life cycle of many parasites such that peak egg production of adult helminths coincides with ecologically relevant events, but these patterns are predominantly recorded through faecal egg counts (e.g. Madhavi, 1979; Rolfe et al., 1991). Although otters are present throughout the year, the observed summer peak in *P. truncatum* fecundity is synchronised with high abundance of the snail hosts (*Bithynia* spp., Dunn, 1978; Lam and Calow, 1989; *Radix balthica*, E. Sherrard-Smith, unpublished data). Although in the current

system host treatment is not a focus, our findings illustrate the importance of identifying the individual and environmental factors that are associated with heavily infected hosts and/or highly fecund parasites. Such detailed knowledge of host–parasite systems could have profound implications for treatment strategies in other systems; a particularly important objective following the emergence of anthelmintic resistance (Laurenson et al., 2013), which could help to prolong the efficacy of drug treatments by ensuring a suitable percentage of parasites are eradicated whilst avoiding the need to blanket treat a host population. Ultimately, this approach contributes to an understanding of the patterns that define parasite populations by highlighting key factors associated with parasite abundance, aggregation and fecundity in this otter-helminth system.

Acknowledgements

We thank Alex Chinchen, funded by a Cardiff University Research Opportunities Program (CUROP), UK, for assistance with egg counting. Samples were provided by the Cardiff University Otter Project funded by the Environment Agency. ES-S was supported by a Natural Environment Research Council Studentship, UK (NER/0329013) with Case Partner, James Williams and the Somerset Otter Group, UK. JC was funded by an Advanced NERC Fellowship, UK (NER/J/S/2002/00706).

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